PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Roy Duncan
Serial No.: to be assigned
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Title: NOVEL REOVIRUS-DERIVED PROTEINS AND USES THEREFOR

Art Unit: 1653
Examiner: to be assigned

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT FOR ENTRY BEFORE CALCULATION OF FILING FEES

Sir:

This is filed as an amendment to the continuation application filed today. Please amend the application as follows:

In the Specification:

Please replace the Title paragraph beginning at page 1, line 1, with the following rewritten paragraph:

--Novel Reovirus-Derived Proteins and Uses Therefor--

Please replace the paragraph beginning at page 6, line 2, with the following rewritten paragraph:

--Figure 1 collectively presents schematic diagrams of reovirus fusion protein-encoding genome segments. Thus, Figure 1A presents segments from Nelson bay virus (NBV); Figure 1B presents segments from avian reovirus (ARV); and Figure 1C presents segments from Baboon Reovirus (BRV).--

Please replace the paragraph beginning at page 7, line 7, with the following rewritten paragraph:

--Exemplary proteins embraced by the above-described profile of properties include proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:2, proteins having an amino acid sequence

substantially the same as set forth in SEQ ID NO:6, proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:10, and the like.--

Please replace the paragraph beginning at page 7, line 14, with the following rewritten paragraph:

--Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID NO:2, proteins having the same amino acid sequence as set forth in SEQ ID NO:6, proteins having the same amino acid sequence as set forth in SEQ ID NO:10, and the like.--

Please replace the paragraph beginning at page 8, line 7, with the following rewritten paragraph:

--Exemplary proteins embraced by the above-described profile of properties include proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:10. Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID NO:14.--

Please replace the paragraph beginning at page 10, line 29, with the following rewritten paragraph:

--Exemplary isolated nucleic acids contemplated for use in the practice of the present invention include nucleic acids having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO:1,

nucleotides 25-1607 of SEQ ID NO:5,

nucleotides 27-1579 of SEQ ID NO:9,

nucleotides 25-832 of SEQ ID NO:13, or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.--

Please replace the paragraph beginning at page 11, line 11, with the following rewritten paragraph:

--Presently preferred isolated and purified nucleic acids, or functional fragments thereof contemplated according to the invention are nucleic acids encoding the above-described proteins, e.g.,

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10 or SEQ ID NO:14, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes biologically active fusion protein.--

Please replace the paragraph beginning at page 11, line 24, with the following rewritten paragraph:

--As employed herein, the term "contiguous nucleotide sequence substantially the same as" refers to DNA having sufficient homology to the reference polynucleotide, such that it will hybridize to the reference nucleotide under typical stringency conditions employed by those of skill in the art. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide encodes substantially the same amino acid sequence of SEQ ID NOs:2, 6, 10 or 14. In another embodiment, DNA having "a contiguous nucleotide sequence substantially the same as" has at least 60% homology with respect to the nucleotide sequence of the reference DNA fragment with which the subject DNA is being compared. In a preferred embodiment, the DNA has at least 70%, more preferably 80%, homology to the comparative nucleotide sequence; with greater than about 90% homology being especially preferred.--

Please replace the paragraph beginning at page 14, line 8, with the following rewritten paragraph:

--As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogues thereof, that has a sequence of nucleotides that includes at least 14, preferably at least 20, more preferably at least 50, contiguous bases that

are the same as (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID NOs:1, 5, 9 or 13. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.--

Please replace the paragraph beginning at page 23, line 6, with the following rewritten paragraph:

--The detergent-protein complexes can be mixed with lipids and the detergent removed by dialysis, chromatography, or extraction according to standard published procedures, similar to methods used to generate influenza HA or Sendai virus F protein-containing virosomes (see Grimaldi, *Res. Virol.*, 146:289-293 (1995) and Ramani et al., *FEBS Lett.*, 404:164-168 (1997)). These procedures will result in the production of proteoliposomes, lipid vesicles containing the ARV, NBV, or BRV fusion proteins embedded in the vesicle membrane. Once again, optimal conditions for proteoliposome production can be empirically determined as can the lipid composition and size of the proteoliposomes which can affect the efficiency of liposome-cell fusion. Bioactive molecules of interest (e.g., nucleic acids, proteins or peptides, pharmacological compounds, and the like) can be included during the formation of the proteoliposomes to facilitate packaging of the molecule within the liposomes. The proteoliposomes can be purified by centrifugation and used to deliver bioactive molecules intracellularly, either in cell culture or *in vivo*, by protein-enhanced fusion of the proteoliposomes with cell membranes.--

Please replace the paragraph beginning at page 27, line 13, with the following rewritten paragraph:

--The two strains of ARV were grown in monolayers of QM5 cells, a continuous quail cell line (see Antin and Ordahl, *Devel.* Biol., 143:111-121 (1991)) while the fusogenic mammalian reoviruses were grown in monkey Vero cells. Virus particles were isolated and concentrated from infected cell lysates by differential centrifugation, as previously described (see Duncan, *Virology*, 219:179-189 (1996)).--

Please replace the paragraph beginning at page 28, line 28, with the following rewritten paragraph:

--The ARV and NBV S1 cDNA clones and the BRV S4 cDNA clone were subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen) under the

control of the CMV promoter. Plasmid DNA was isolated and purified on Qiagen midi columns (Qiagen) according to the manufacturer's specifications. Plasmid DNA (1 µg) was mixed with Lipofectamine (3 µl) (Life Technologies Inc.) and used to transfect sub-confluent cell monolayers grown in 12 well cluster plates. Transfected cell monolayers were incubated at 37°C for 24-48 hr before being fixed with methanol and stained using a water-soluble Wright-Giemsa stain (DiffQuik; VWR-Canlab) or by immunostaining using viral-specific antiserum obtained from infected animals, as previously described (see Duncan et al., *Virology*, 224:453-464 (1996)). Cell fusion was assessed by light microscopy of stained monolayers and syncytial foci were photographed at 100x magnification.--

Please replace the paragraph beginning at page 29, line 17, with the following rewritten paragraph:

--Sequence analysis determined that the ARV and NBV S1 genome segments contained three sequential overlapping open reading frames (ORFs) while the BRV S4 genome segment contained 2 ORFs. In order to determine which ORF encoded the viral fusion protein, portions of these genome segments were subcloned into pcDNA3 by PCR amplification of individual regions using sequence-specific primers as indicated in the figures. The subcloned regions were analyzed for their fusion-inducing ability by transfection analysis as described above.--

Please replace the paragraph beginning at page 29, line 29, with the following rewritten paragraph:

--Two unrelated fusion proteins responsible for the cell-cell fusion induced by avian reovirus (ARV) and the only two fusogenic mammalian reoviruses, Nelson Bay virus (NBV) and baboon reovirus (BRV) have been identified. These proteins are referred to herein as P11 (for ARV and NBV) and P15 (for BRV) to reflect their approximate predicted molecular weights. The genes encoding P11 from two strains of ARV (strain 176 and strain 138) and from NBV have been cloned and sequenced, as has the gene from BRV that encodes P15. The sequence-predicted structural organization of these proteins has been analyzed, and the membrane fusion properties thereof have been directly demonstrated.--

Please replace the paragraph beginning at page 32, line 6, with the following rewritten paragraph:

--The ARV and NBV P11 proteins are small proteins (98 or 95 amino acids, respectively) that share approximately 33% sequence homology and a similar domain organization indicating that these proteins are evolutionarily related (Figure 2). Both proteins lack obvious signal peptides, suggesting that they insert in membranes post-translationally. Both proteins also contain one predicted transmembrane domain located in the central portion of the protein resulting in small (approximately 40 amino acid) intracellular and extracellular domains. The conserved clustering of positively charged amino acids on the carboxy-proximal side of the transmembrane domain is consistent with the amino-terminal domain residing extracellularly (von Heijne, *Curr. Op. Cell Biol.*, 2:604-608 (1990)). The four cysteine residues in each protein are conserved, suggesting that the ARV and NBV P11 proteins assume a similar tertiary and quaternary structure. The ARV P11 protein is devoid of N-linked glycosylation sites, implying that post-translational glycosylation is not required for functional protein folding, a prediction that has been confirmed experimentally (see Duncan et al. (1996), *supra*). Although the NBV P11 protein contains a single potential N-linked glycosylation site, this site is probably not glycosylated since inhibitors of glycosylation fail to affect NBV-induced cell fusion (see Wilcox and Compans (1983)). The size, absence of signal peptides, and N-linked glycosylation, and predicted domain organization of the ARV and NBV P11 proteins clearly distinguishes these proteins from the well characterized enveloped virus fusion proteins and suggests that P11 represents a novel type of membrane fusion protein.--

Please replace the paragraph beginning at page 33, line 24, with the following rewritten paragraph:

--The fusion-inducing potential of these reovirus proteins has been directly demonstrated by expressing them in transfected cells in the absence of any other reovirus proteins; intracellular expression triggers the induction of cell-cell fusion and syncytium formation characteristic of virus infection by this group of fusogenic reoviruses. Thus, quail cell monolayers were mock transfected, or transfected with plasmid DNA expressing the ARV, BRV, or NBV fusion proteins. Transfected cells were fixed and the nuclei stained using a Wright-Giemsa stain at 36 hr post infection and the stained monolayers were photographed at 100x magnification.--

In the Claims:

Please cancel claims 1 to 3 and 29 to 40.

Claims 5 to 28 have been amended and claims 43 to 77 have been added. Claims 4 to 28 and 41 to 77 are now pending.

Amended claims 5 to 28 and new claims 43 to 79 are as follows:

5. [Amended] The protein of claim 4 having an amino acid sequence substantially the same as set forth in SEQ ID NO: 14 [BRV].

6. [Amended] The protein of claim 4 having the amino acid sequence set forth in SEQ ID NO: 14 [BRV].

7. [Amended] An antibody raised against the protein of claim 43.

8. [Amended] An antibody raised against the protein of claim 4.

9. [Amended] An isolated nucleic acid encoding the protein of claim 43.

10. [Amended] An isolated nucleic acid according to claim 9 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO: 1 [ARV1],

nucleotides 25-1607 of SEQ ID NO: 5 [ARV2],

nucleotides 27-1579 of SEQ ID NO: 9 [NBV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

11. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim 43, wherein the nucleic acis is selected from the group consisting of:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 6 or SEQ ID NO: 10, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.

12. [Amended] An isolated nucleic acid according to claim 9 operatively associated with an inducible promoter.

13. [Amended] An isolated nucleic acid encoding the protein of claim 4.

14. [Amended] The isolated nucleic acid of claim 13 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-832 of SEQ ID NO: 13 [BRV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

15. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim 4, wherein the nucleic acid is selected from the group consisting of:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO: 14, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.

16. [Amended] The isolated nucleic acid of claim 13 operatively associated with an inducible promoter.

17. [Amended] A cell containing the protein of claim 43.

18. [Amended] The cell containing the protein of claim 4.

19. [Amended] The cell containing the nucleic acid of claim 9.

20. [Amended] The cell containing the nucleic acid of claim 12.

21. [Amended] The cell containing the nucleic acid of claim 13.

22. [Amended] The cell containing the nucleic acid of claim 16.

23. [Amended] Liposomes containing the protein of claim 43.

24. [Amended] Liposomes containing the protein of claim 4.

25. [Amended] Liposomes containing the nucleic acid of claim 9.

26. [Amended] Liposomes containing the nucleic acid of claim 13.

27. [Amended] A method for producing the protein of claim 43, said method comprising the step of expressing a nucleic acid encoding said protein in a suitable host.

28. [Amended] A method for producing the protein of claim 4, said method comprising the step of expressing a nucleic acid encoding said protein in a suitable host.

43. [New] An isolated protein which:
(a) is a membrane fusion protein;
(b) comprises a transmembrane domain; and
(c) has at least 33% amino acid sequence identity to a protein which:
    (i) is encoded by a polynucleotide from the genome of Reoviridae;
    (ii) is a membrane fusion protein, and
    (iii) has a molecular weight of about 11 kDa.

44. [New] The isolated protein of claim 43 which:
(a) is encoded by a polynucleotide from the genome of Reoviridae; and
(b) has molecular weight of about 11 kDa.

45. [New] The protein of claim 43 which has less than 100 amino acids or has about 100 amino acids.

46. [New] The protein of claim 43 which contains a cluster of positive amino acid residues, wherein the cluster is located on the C-terminal side of the

transmembrane domain and comprises at least 4 positive residues within the 20 residues flanking the transmembrane domain at the C-terminal side.

47. [New] The protein of claim 43 which lacks a signal peptide.

48. [New] The protein of claim 43 which contains 4 cysteine residues at conserved positions relative to SEQ ID NO:2 (ARV1); SEQ ID NO:6 (ARV2); and SEQ ID NO:10 (NBV).

49. [New] The isolated protein of claim 43 which has at least 33% amino acid sequence identity to a polypeptide selected from the group consisting of:
(a) a polypeptide of SEQ ID NO:2 (ARV1);
(b) a polypeptide of SEQ ID NO:6 (ARV2); and
(c) a polypeptide of SEQ ID NO:10 (NBV).

50. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:2 (ARV1).

51. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:6 (ARV2).

52. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:10 (NBV).

53. [New] The protein of claim 49 which comprises a cluster of positive amino acid residues, wherein the cluster is located on the C-terminal side of the transmembrane domain and comprises at least 4 positive residues within the 20 residues flanking the transmembrane domain at the C-terminal side.

54. [New] The protein of claim 49 which lacks a signal peptide.

55. [New] The protein of claim 49 which comprises 4 cysteine residues at conserved positions relative to SEQ ID NO:2 (ARV1); SEQ ID NO:6 (ARV2); and SEQ ID NO:10 (NBV).

56. [New] An isolated protein comprising the sequence selected from the group consisting of: SEQ ID NO:2(ARV1), SEQ ID NO:6(ARV2) and SEQ ID NO:10(NBV).

57. [New] An isolated protein which:

(a) is a membrane fusion protein;
(b) comprises a transmembrane domain;
(c) has a molecular weight of about 15 kDa; and
(d) is encoded by a polynucleotide from the genome of Reoviridae.

58. [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with the protein of claim 43 for a time and under conditions effective to promote membrane fusion.

59. [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with the protein of claim 57 for a time and under conditions effective to promote membrane fusion.

60. [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with a membrane fusion protein for a time and under conditions effective to promote membrane fusion, wherein the membrane fusion protein is encoded by a polynucleotide of the genome of a fusogenic member of the family Reoviridae or is substantially the same as the membrane fusion protein encoded by a polynucleotide of the genome of a fusogenic member of the family Reoviridae.

61. [New] The method of claim 60 wherein the fusogenic member of the family Reoviridae is selected from: ARV, NBV and BRV.

62. [New] The method of claim 60 wherein the membranes are cell membranes, liposome membranes or proteoliposome membranes.

63. [New] The method of claim 60 wherein the membranes are the cell membrane of an immortalized myeloma cell and the cell membrane of a primary B cell or T cell.

64. [New] The method of claim 63 wherein the immortalized myeloma cell is human or mouse, and wherein the primary B cell or T cell is a purified spleen cell from an immunized mammal.

65. [New] The method of claim 60 wherein the membranes to be fused are the cell membrane of an immortalized myeloma cell and the cell membrane of a primary B cell or T cell, and wherein the membrane fusion protein has an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10, 14, and substantially the same sequences thereof.

66. [New] A method to promote membrane fusion between a first membrane and a second membrane, said method comprising:
(a) introducing a fusogenic protein, in an amount sufficient to effect membrane fusion, into the first membrane; and then,
(b) contacting the second membrane with the first membrane for a time and under conditions effective to promote membrane fusion between the first membrane and the second membrane;

wherein the first membrane is selected from the group consisting of:
(i) a liposome membrane;
(ii) a proteoliposome membrane; and
(iii) a membrane of a cell;

and wherein the fusogenic protein either:
(i) is encoded by a polynucleotide of the genome of Reoviridae; and
(ii) has a molecular weight of about 11 kDa; and
(iii) is less than 100 amino acids or is about 100 amino acids;
or wherein the fusogenic protein:
(i) is encoded by a polynucleotide of the genome of Reoviridae; and
(ii) has a molecular weight of about 15 kDa; and
(iii) is less than 150 amino acids or is about 150 amino acids.

67. [New] The method according to claim 66 wherein the first membrane is the liposome membrane or the proteoliposome membrane.

68. [New] The method according to claim 67, wherein the step of introducing the fusogenic protein comprises incorporating the fusogenic protein into the liposome membrane or the proteoliposome membrane.

69. [New] The method according to claim 66 wherein the first membrane is the cell membrane.

70. [New] The method according to claim 69, wherein the step of introducing the fusogenic protein comprises the step of introducing into the cell an expression vector comprising a polynucleotide which encodes the fusogenic protein, wherein the vector is free of full-length reovirus genome.

71. [New] A method to promote membrane fusion between a first membrane and a second membrane, said method comprising contacting the first or

second membrane or both membranes with an effective amount of a protein-liposome complex for a time and under conditions effective to promote membrane fusion between the first membrane and the second membrane, wherein the protein-liposome complex contains a fusogenic protein; and

wherein the fusogenic protein either:
(i) is encoded by a polynucleotide of the genome of Reoviridae;
(ii) has a molecular weight of about 11 kDa; and
(iii) is less than 100 amino acids or is about 100 amino acids;
or wherein the fusogenic protein:
(i) is encoded by a polynucleotide of the genome of Reoviridae;
(ii) has a molecular weight of about 15 kDa; and
(iii) is less than 150 amino acids or is about 150 amino acids.

72. [New] The method according to claim 66 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

73. [New] The method according to claim 67 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

74. [New] The method according to claim 68 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

75. [New] The method according to claim 69 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

76. [New] The method according to claim 70 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

77. [New] The method according to claim 71 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.

REMARKS

The Title of the invention has been amended for clarity.

References to SEQ ID NOs: 7, 8, 9 and 10 have been replaced with SEQ ID NOs: 9, 10, 13 and 14 respectively. The amendments correct the references to amino acid and nucleic acid sequences, as set forth in the Sequence Listing. Sequence numbering is now consistent throughout the specification.

The amendments to pages 6 and 30 correct clerical errors. The amendments are supported at Figure 1.

The amendments to pages 23, 27, 29 and 33 correct typographical errors.

The amendment to page 32 corrects a typographical error. The amendment is supported at Figure 2, which shows 33 positions of identity out of a total of (approximately) 100 residues, among the amino acid sequences of the P11 proteins of ARV-138, ARV-176 and NBV.

Claims 5 to 28 have been amended for further clarity and to correct the references to amino acid and nucleic acid sequences, as set forth in the Sequence Listing.

Applicant points out that this is the first time that fusion-inducing proteins have been discovered in nonenveloped viruses and represent a novel class of fusion proteins. Therefore, the claimed proteins and methods recite the feature that the protein is encoded by, or is related to, the genome of *Reoviridae*. As claimed, the proteins of the present invention are distinguished from known fusion proteins, notably those of enveloped viruses.

With respect to new claims 43 to 45 and 49 to 52, proteins related to those encoded by *Reoviridae* are defined by the functional characteristic that the proteins have membrane fusion activity and by two structural characteristics: a minimum 33% amino acid sequence homology to the protein encoded by the genome of *Reoviridae* and a transmembrane domain. The defining feature "33% amino acid sequence identity" is supported at page 32, line 6, as corrected above. The relationship among the amino acid sequences of the P11 proteins of ARV-138, ARV-176 and NBV is such that a minimum of 33% homology sufficiently defines the variant, and is within the scope of the

invention as long as the variant contains a transmembrane domain and has membrane fusion activity. Fusion activity can be readily detected by applying a simple test, such as that exemplified in Example 4 (page 28 of the specification).

New claims 46 and 53 recite "*a cluster of positive amino acid residues, wherein the cluster is located on the C-terminal side of the transmembrane domain and comprises at least 4 positive residues within the 20 residues flanking the transmembrane domain at the C-terminal side*". These claims are supported at least at page 32, lines 16 to 20, as well as Figure 2. The expression "at least" is supported by Figure 2, which shows that there may be positive residues (K, R and possibly H) within the cluster in addition to the 6 residues of the consensus sequence.

New claims 47 and 54 recite that the protein "*lacks a signal peptide*". This feature is supported at least by claim 1 and Example 7.

New claims 48 and 55 recite "4 cysteine residues at conserved positions relative to SEQ ID NO:2 (ARV1); SEQ ID NO:6 (ARV2); and SEQ ID NO:10 (NBV)". Support for this feature is found at least at page 32, lines 20 to 23, as well as Figure 2.

New claim 56 is supported at least at claim 2.

New claim 57 is supported at least at claim 4 and, as explained above, the claim recites the feature that the protein is encoded by a polynucleotide from the genome of *Reoviridae*.

New claim 58 to 77 are drawn to methods of using fusogenic proteins of Reoviridae or related proteins to fuse membranes. The claims are supported throughout the specification and the claims as originally filed.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached page is captioned "**Version with markings to show changes made**".

No new matter is introduced by the amendments or by the new claims.

The Petition for Extension of Time pursuant to 37 CFR 1.136(a) and the fee have been submitted with our letters of June 29 and July 30, 2001. The

U.S.P.T.O. is authorized to withdraw from our deposit account number 19-2550 any excess claims fees if required.

Respectfully submitted,

Date: Aug 30, 2001

Thuy H. Nguyen
Registration No. P-47336
Telephone: (613) 232-2468
Facsimile: (613) 232-8440

SMART & BIGGAR
900-55 Metcalfe Street
Ottawa, Ontario
Canada, K1P 5Y6

In re Application of:
Roy Duncan
Application No.: to be assigned
Filed: Continuation of SN 08/965,708
filed November 7, 1997

PATENT
Attorney Docket No. 78973-1C

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The Title paragraph beginning at page 1, line 1, has been amended as follows:

Novel ~~Reovirus-derived Proteins, Nucleic Acids Encoding Same,~~Reovirus-Derived Proteins and Uses Therefor

The paragraph beginning at page 6, line 2, has been amended as follows:

Figure 1 collectively presents schematic diagrams of reovirus fusion protein-encoding genome segments. Thus, Figure 1A presents segments from ~~avian reovirus (ARV);~~Nelson bay virus (NBV); Figure 1B presents segments from ~~Nelson bay virus~~avian reovirus ~~(NBV);~~(ARV); and Figure 1C presents segments from Baboon Reovirus (BRV).

The paragraph beginning at page 7, line 7, has been amended as follows:

Exemplary proteins embraced by the above-described profile of properties include proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:2, proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:6, proteins having an amino acid sequence substantially the same as set forth in SEQ ID ~~NO:8,~~NO:10, and the like.

The paragraph beginning at page 7, line 14, has been amended as follows:

Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID NO:2, proteins having the same amino acid sequence as set forth in SEQ ID NO:6, proteins having the same amino acid sequence as set forth in SEQ ID ~~NO:8,~~NO:10, and the like.

The paragraph beginning at page 8, line 7, has been amended as follows:

Exemplary proteins embraced by the above-described profile of properties include proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:10. Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID ~~NO:10.~~NO:14.

The paragraph beginning at page 10, line 29, has been amended as follows:

Exemplary isolated nucleic acids contemplated for use in the practice of the present invention include nucleic acids having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO:1,

nucleotides 25-1607 of SEQ ID NO:5,

nucleotides 27-1579 of SEQ ID ~~NO:7,~~NO:9,

nucleotides 25-832 of SEQ ID ~~NO:9,~~NO:13, or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

The paragraph beginning at page 11, line 11, has been amended as follows:

Presently preferred isolated and purified nucleic acids, or functional fragments thereof contemplated according to the invention are nucleic acids encoding the above-described proteins, e.g.,

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:6, SEQ ID ~~NO:8 or SEQ ID NO:10,~~NO:10 or SEQ ID NO:14, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes biologically active fusion protein.

The paragraph beginning at page 11, line 24, has been amended as follows:

As employed herein, the term "contiguous nucleotide sequence substantially the same as" refers to DNA having sufficient homology to the reference polynucleotide, such that it will hybridize to the reference nucleotide under typical stringency conditions employed by those of skill in the art. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide encodes substantially the same amino acid sequence of SEQ ID NOs:2, 6, ~~8~~10 or ~~10.~~14. In another embodiment, DNA having "a contiguous nucleotide sequence substantially the same as" has at least 60% homology with respect to the nucleotide

sequence of the reference DNA fragment with which the subject DNA is being compared. In a preferred embodiment, the DNA has at least 70%, more preferably 80%, homology to the comparative nucleotide sequence; with greater than about 90% homology being especially preferred.

The paragraph beginning at page 14, line 8, has been amended as follows:

As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogues thereof, that has a sequence of nucleotides that includes at least 14, preferably at least 20, more preferably at least 50, contiguous bases that are the same as (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID NOs:1, 5, ~~7~~9 or ~~9~~13. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.

The paragraph beginning at page 23, line 6, has been amended as follows:

The detergent-protein complexes can be mixed with lipids and the detergent removed by dialysis, chromatography, or extraction according to standard published procedures, similar to methods used to generate influenza HA or Sendai virus F protein-containing virosomes (see Grimaldi, *Res. Virol.*, 146:289-293 (1995) and Ramani et al., *FEBS Lett.*, 404:164-168 (1997)). These procedures will result in the production of proteoliposomes, lipid vesicles containing the ARV, NBV, or BRV fusion proteins embedded in the vesicle membrane. Once again, optimal conditions for proteoliposome production can be empirically determined as can the lipid composition and size of the proteoliposomes which can affect the efficiency of liposome-cell fusion. Bioactive molecules of interest (e.g., nucleic acids, proteins or peptides, pharmacological compounds, and the like) can be included during the formation of the proteoliposomes to facilitate packaging of the molecule within the liposomes. The proteoliposomes can be purified by centrifugation and used to deliver bioactive molecules intracellularly, either in cell culture or *in vivo*, by protein-enhanced fusion of the proteoliposomes with cell membranees.

The paragraph beginning at page 27, line 13, has been amended as follows:

The two strains of ARV were grown in monolayers of QM5 cells, a continuous quail cell line (see Antin and Ordahl, *Devel.* Biol., 143:111-121 (1991)) while the fusogenic mammalian reoviruses were grown in monkey Vero cells. Virus particles were isolated and concentrated from infected cell lysates by differential centrifugation, as previously described (see Duncan, *Virology*, 219:179-189 (1996)).

The paragraph beginning at page 28, line 28, has been amended as follows:

The ARV and NBV S1 cDNA clones and the BRV S4 cDNA clone were subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen) under the control of the CMV promoter. Plasmid DNA was isolated and purified on Qiagen midi columns (Qiagen) according to the manufacturer's specifications. Plasmid DNA (1 μg) was mixed with Lipofectamine (3 μl) (Life Technologies Inc.) and used to transfect sub-confluent cell monolayers grown in 12 well cluster plates. Transfected cell ~~monolyaers~~monolayers were incubated at 37°C for 24-48 hr before being fixed with methanol and stained using a water-soluble Wright-Giemsa stain (DiffQuik; VWR-Canlab) or by immunostaining using viral-specific antiserum obtained from infected animals, as previously described (see Duncan et al., *Virology*, 224:453-464 (1996)). Cell fusion was assessed by light microscopy of stained monolayers and syncytial foci were photographed at 100x magnification.

The paragraph beginning at page 29, line 17, has been amended as follows:

Sequence analysis determined that the ARV and NBV S1 genome segments contained three sequential overlapping open reading frames (ORFs) while the BRV S4 genome segment contained 2 ORFs. In order to determine which ORF encoded the viral fusion protein, portions of these genome segments were subcloned into pcDNA3 by PCR amplification of individual regions using sequence-specific primers as indicated in the figures. The subcloned regions were analyzed for their fusion-inducing ability by transfection analysis as described above.

The paragraph beginning at page 29, line 29, has been amended as follows:

Two unrelated fusion proteins responsible for the cell-cell fusion induced by avian reovirus (ARV) and the only two fusogenic mammalian reoviruses, Nelson Bay virus (NBV) and baboon reovirus (BRV) have been identified. These proteins are referred to herein as P11 (for ARV and NBV) and P15 (for BRV) to reflect their approximate predicted molecular weights. The genes encoding P11 from two strains of ARV (strain 176 and strain 138) and from NBV have been cloned and sequenced, as has the gene from ~~NBV~~BRV that encodes P15. The sequence-predicted structural organization of these proteins has been analyzed, and the membrane fusion properties thereof have been directly demonstrated.

The paragraph beginning at page 33, line 24, has been amended as follows:

The fusion-inducing potential of these reovirus proteins has been directly demonstrated by expressing them in transfected cells in the absence of any other reovirus proteins; intracellular expression triggers the induction of cell-cell fusion and syncytium formation characteristic of virus infection by this group of fusogenic reoviruses. Thus, ~~quali~~quail cell monolayers were mock transfected, or transfected with plasmid DNA expressing the ARV, BRV, or NBV fusion proteins. Transfected cells were fixed and the nuclei stained using a Wright-Giemsa stain at 36 hr post infection and the stained monolayers were photographed at 100x magnification.

The paragraph beginning at page 32, line 6, has been amended as follows:

The ARV and NBV P11 proteins are small proteins (98 or 95 amino acids, respectively) that share approximately ~~38%~~33% sequence homology and a similar domain organization indicating that these proteins are evolutionarily related (Figure 2). Both proteins lack obvious signal peptides, suggesting that they insert in membranes post-translationally. Both proteins also contain one predicted transmembrane domain located in the central portion of the protein resulting in small (approximately 40 amino acid) intracellular and extracellular domains. The conserved clustering of positively charged amino acids on the carboxy-proximal side of the transmembrane domain is consistent with the amino-terminal domain residing extracellularly (von Heijne, *Curr. Op. Cell Biol.*, 2:604-608 (1990)). The four cysteine residues in each protein are conserved, suggesting that the ARV and NBV P11 proteins assume a similar tertiary and quaternary structure. The ARV P11 protein is devoid of N-linked glycosylation sites, implying that post-translational glycosylation is not required for functional protein folding, a prediction that has been confirmed experimentally (see Duncan et al. (1996), *supra*). Although the NBV P11 protein contains a single potential N-linked glycosylation site, this site is probably not glycosylated since inhibitors of glycosylation fail to affect NBV-induced cell fusion (see Wilcox and Compans (1983)). The size, absence of signal peptides, and N-linked glycosylation, and predicted domain organization of the ARV and NBV P11 proteins clearly distinguishes these proteins from the well characterized enveloped virus fusion proteins and suggests that P11 represents a novel type of membrane fusion protein.

In the Claims:

Claims 5 to 28 have been amended as follows:

5. ~~A protein according to~~[Amended] The protein of claim 4 having an amino acid sequence substantially the same as set forth in SEQ ID ~~NO 10~~NO: 14 [BRV].

6. ~~A protein according to~~[Amended] The protein of claim 4 having the amino acid sequence set forth in SEQ ID NO: ~~10~~14 [BRV].

7. [Amended] An antibody raised against the protein of claim ~~1.~~43.

8. [Amended] An antibody raised against the protein of claim 4.

9. [Amended] An isolated nucleic acid encoding ~~protein according to claim 1.~~the protein of claim 43.

10. [Amended] An isolated nucleic acid according to claim 9 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO: 1 [ARV1],

nucleotides 25-1607 of SEQ ID NO: 5 [ARV2],

nucleotides 27-1579 of SEQ ID NO: ~~7~~9 [NBV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

11. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim ~~1,~~43, wherein the nucleic acis is selected from the group consisting of:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 6 or SEQ ID NO: ~~8,~~10, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.

12. [Amended] An isolated nucleic acid according to claim 9 operatively associated with an inducible promoter.

13. [Amended] An isolated nucleic acid encoding ~~protein according to~~the protein of claim 4.

14. ~~An~~[Amended] The isolated nucleic acid ~~according to~~of claim 13 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-832 of SEQ ID NO: ~~9~~13 [BRV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

15. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim 4, wherein the nucleic acid is selected from the group consisting of:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO: ~~10,~~14, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.

16. ~~An~~[Amended] The isolated nucleic acid ~~according to~~of claim 13 operatively associated with an inducible promoter.

17. ~~Cells containing protein according to claim 1.~~

~~18. Cells containing protein according to claim 4.~~

~~19. Cells containing nucleic acid according to claim 9.~~

~~20. Cells containing nucleic acid according to claim 12.~~

~~21. Cells containing nucleic acid according to claim 13.~~

~~22. Cells containing nucleic acid according to claim 16.~~

~~23. Liposomes containing protein according to claim 1.~~

~~24. Liposomes containing protein according to claim 4.~~

~~25. Liposomes containing nucleic acid according to claim 9.~~

~~26. Liposomes containing nucleic acid according to~~[Amended] A cell containing the protein of claim 43.

18. [Amended] The cell containing the protein of claim 4.

19. [Amended] The cell containing the nucleic acid of claim 9.

20. [Amended] The cell containing the nucleic acid of claim 12.

21. [Amended] The cell containing the nucleic acid of claim 13.

22. [Amended] The cell containing the nucleic acid of claim 16.

23. [Amended] Liposomes containing the protein of claim 43.

24. [Amended] Liposomes containing the protein of claim 4.

25. [Amended] Liposomes containing the nucleic acid of claim 9.

26. [Amended] Liposomes containing the nucleic acid of claim 13.

27. ~~A method for the production of protein according to claim 1, said method comprising expressing~~[Amended] A method for producing the protein of claim 43, said method comprising the step of expressing a nucleic acid encoding said protein in a suitable host.

28. ~~A method for the production of protein according to~~[Amended] A method for producing the protein of claim 4, said method comprising the step of expressing a nucleic acid encoding said protein in a suitable host.